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THE DIGESTIBILITY OF BUR CLOVER AS AFFECTED BY EXPOSURE TO SUNLIGHT AND RAIN

H. R. GUILBERT[1] AND S. W. MEAD[2]

The principal forage plants of California foothill and valley ranges are annuals.[3] They germinate with the coming of the fall rains and make, during the winter, an amount of growth that varies according to moisture and temperature conditions. From February to May is usually the period of greatest growth. When the rains cease and moisture is depleted, the forage matures and dries. Stock is then either maintained on the dry feed or moved to summer ranges in the high mountains. In the latter case, the stock is brought to the lower ranges in the early fall and subsists on the old dry feed until rains bring on new forage.

The changes in the plants from the early vegetative stage to the dried condition involve marked changes in chemical composition and nutritive value. After drying, the feed is subjected to the processes of weathering.

Studies by Woodman and others[1, 2, 3] on the nutritive value of pasture have shown that young pasture grass is in digestible composition a "watered concentrate" rather than a roughage. They found that 70 per cent of the organic matter was digestible and that the small amount of fiber which it contained was 80 per cent digestible. The immature grass contained approximately 20 per cent digestible protein with a nutritive ratio of about 1:3. As plants approach

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[3] The most common of the grasses are several species of brome, wild oats (*Avena fatua*), fescue grass (*Festuca megalura*), and foxtail (*Hordeum murinum*). Bur clover (*Medicago hispida*) and alfilaria (*Erodium* sp.) are found on the better ranges. Salt grass (*Distichlis spicata*), *a perennial*, is also important in some areas.

maturity, the percentage of nitrogen-free extract and fiber increases, while the protein decreases. The result is a widening of the nutritive ratio and a decrease in digestibility.

Cattle do not graze extensively on green bur clover when other forage such as grasses and alfilaria are present in adequate amounts. As soon as the forage matures and dries, however, they show a decided preference for bur clover.

A high forage value is generally recognized in bur clover ranges during the first few weeks after the feed has cured, and the final finish is usually made by fattening cattle during this period. A late rain, coming after the feed is cured, is disastrous from the standpoint of finishing cattle without supplement; and under such conditions it has, in many cases, been found difficult even to maintain breeding stock.

The most efficient utilization of range forage is a problem involving the proper supplementing of this feed in such a way as to keep the animals supplied at all times with a well balanced diet. To accomplish this it is necessary to have definite information on the changes in composition of the feed and its effect on the nutrition of the animal. Since bur clover is an important forage species which maintains good feeding qualities in the cured condition, the effect of weathering upon its nutritive value is of particular interest.

DIGESTION EXPERIMENTS WITH BUR CLOVER

Approximately one acre of a nearly pure stand of bur clover was cut May 17, 1927, the only contamination being a few star thistles. Most of the burs were still green at the time of cutting, but the seeds were well formed and most of them were yellow in color after the forage had been dried.

Immediately after cutting, the clover was spread out in a thin layer on a clean, concrete pavement and exposed to direct sunlight. It dried rapidly, and on the following day a portion was piled in small cocks for further curing. On the fourth day a rain storm, lasting a few minutes, necessitated the placing of this material in larger piles to minimize wetting. Only the tops and bottoms of these piles were wet, and apparently the shower did little damage. The following day the clover was turned and allowed to cure in large cocks until the seventh day after cutting, at which time it was chopped, thoroughly mixed, sacked, and stored in a dark loft. This portion was bright green in color and was designated as bur clover No. 1.

The remainder of the clover was allowed to dry and bleach in the sun. It was spread out in a thin layer and was mixed and turned at intervals of a few days in order to expose all the material to the sun and simulate field conditions as nearly as possible. During this period (May 17 to June 7) two showers totaling 0.31 of an inch of rain fell. On June 7 another rain storm threatened. One-half of the clover was therefore put under cover and later chopped, mixed, sacked, and stored. This was designated as bur clover No. 2. In contrast to bur clover No. 1, it was brown in color.

This second rain, which amounted to 0.47 inch, fell on the clover remaining after lot 2 had been removed. The water which drained from the clover was decidedly brown in color. The leaching effect of this rain was more noticeable than that of the two previous lighter showers. After the rain, this material was left exposed for an additional 14 days; then it was chopped, mixed, sacked, and stored. It was designated as bur clover No. 3. The treatment of each of the lots may be briefly summarized as follows:

Bur clover No. 1. Cured for one day in a thin layer, then cured in cocks for six days.

Bur clover No. 2. Exposed in a thin layer for 21 days, during which time it was wet twice by rain totaling 0.31 inch.

Bur clover No. 3. Exposed in a thin layer for 34 days, during which time it was wet three times by rain totaling 0.78 inch.

The original plan was to study the influence of varying periods of exposure to sunlight, upon digestibility. The late rains provided an opportunity to study the influence of this additional factor upon the feed, but it was unfortunate that the study of lot 2 was complicated by wetting, as it eliminated the possibility of comparing directly the relative influence of exposure to sunlight and to rain.

During March, April, and May, 1928, digestion experiments were conducted on these three lots of bur clover. The same five wether sheep were used in each of the trials. They were fed an amount of bur clover which was calculated to be sufficient for maintenance, the value assumed being similar to that of average alfalfa hay. The methods used in conducting these experiments were fully discussed in an earlier publication[4] and are summarized here.

The animals were placed in individual box stalls 4 feet by 8 feet, equipped with mangers so constructed as to prevent any possible loss of feed. The feces were collected by means of rubber-lined sacks attached to each animal.

The preliminary feeding period was 10 days and the collection period 15 days.

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The animals were placed in individual box stalls 4 feet by 8 feet, equipped with mangers so constructed as to prevent any possible loss of feed. The feces were collected by means of rubber-lined sacks attached to each animal.

The preliminary feeding period was 10 days and the collection period 15 days.

An amount of bur clover sufficient to last throughout a digestion trial was thoroughly mixed and spread out on a clean concrete floor. The individual feeds for the entire period were then weighed out into paper bags. The bags were labeled designating the animal to which the feed contained was to be given. To obtain a sample for chemical analysis a large quantity was taken and reduced to about ¼ bushel by mixing and quartering. This amount was then ground in a hammer mill, thoroughly mixed, and the final sample for chemical analysis taken from the fine material. A sample for moisture determination was taken before grinding.

The collection bags were emptied twice daily. The feces were immediately weighed and aliquot portions of the feces of each animal were placed in glass mason jars which had been previously rinsed in a 10 per cent alcoholic thymol solution. In addition, powdered thymol was sprinkled over the feces after they were transferred from the scales to the jar to the amount of 5 grams to each jar. The jars were immediately placed in a refrigerator where they were maintained at a temperature varying from 28 to 35 degrees Fahrenheit. At the end of the collection period the contents of the several jars representing the total feces collected from each animal were thoroughly mixed, ground, remixed and sampled for chemical analysis.

The data from the digestion trial with bur clover No. 1 are given in tables 1, 2, and 3.

TABLE 1

TOTAL FEED CONSUMED AND TOTAL FECES COLLECTED

Sheep No.	Bur clover No. 1, grams	Feces grams
137	9,600	7,457.5
139	12,000	9,722.5
717	10,500	8,589.5
138	7,500	5,349.5
135	11,700	10,556.0

TABLE 2

CHEMICAL ANALYSES OF FECES AND OF BUR CLOVER NO. 1

Feces	Dry matter per cent	Crude protein per cent	Nitrogen-free extract per cent	Ether extract per cent	Crude fiber per cent
Sheep No. 137	38.85	5.00	13.88	1.63	12.72
Sheep No. 139	38.01	4.68	14.73	1.60	11.56
Sheep No. 717	38.46	5.22	14.43	1.70	11.69
Sheep No. 138	43.34	5.63	17.15	1.66	13.11
Sheep No. 135	35.12	4.32	13.71	1.21	11.19
Bur Clover No. 1	86.62	15.34	40.65	2.89	19.90

TABLE 3

COEFFICIENTS OF DIGESTIBILITY OF BUR CLOVER NO. 1

Sheep No.	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber
137	65.16	74.68	73.48	56.19	50.35
139	64.45	75.18	70.64	55.14	52.93
717	63.68	72.27	70.96	51.88	51.94
138	64.31	73.82	69.91	59.03	53.07
135	63.42	74.59	69.57	62.22	49.27
Average	*64.20*	*74.11*	*70.91*	*56.89*	*51.51*

The data from the digestion trial with bur clover No. 2 are given in tables 4, 5, and 6.

TABLE 4

TOTAL FEED CONSUMED AND TOTAL FECES COLLECTED

Sheep No.	Bur clover No. 2 grams	Feces, grams
137	8,353.0	7,072.0
139	11,866.0	10,355.0
717	9,667.0	8,398.5
138	7,387.0	5,996.5
135	11,491.0	10,300.0

TABLE 5

CHEMICAL ANALYSES OF FECES AND OF BUR CLOVER NO. 2

Feces	Dry matter per cent	Crude protein per cent	Nitrogen-free extract per cent	Ether extract per cent	Crude fiber per cent
Sheep No. 137	41.16	5.39	16.93	1.47	12.69
Sheep No. 139	40.28	5.36	16.57	1.48	12.22
Sheep No. 717	41.97	6.28	16.88	1.58	12.22
Sheep No. 138	44.66	6.03	17.89	1.66	13.82
Sheep No. 135	39.98	5.23	16.28	1.46	12.22
Bur Clover No. 2	87.48	15.18	40.48	2.22	21.97

TABLE 6

COEFFICIENTS OF DIGESTIBILITY OF BUR CLOVER NO. 2

Sheep No.	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber
137	60.17	69.88	64.59	43.91	51.10
139	59.82	69.19	64.28	41.80	51.46
717	58.32	64.06	63.77	38.16	51.68
138	58.56	67.75	64.12	39.33	48.94
135	59.04	69.12	63.95	41.04	50.14
Average	*59.18*	*68.00*	*64.14*	*40.85*	*50.66*

The data from the digestion trial with bur clover No. 3 are shown in tables 7, 8, and 9.

TABLE 7

TOTAL FEED CONSUMED AND TOTAL FECES COLLECTED

Sheep No.	Bur clover No. 3 grams	Feces, grams
137	8,700.0	8,181.0
139	12,000.0	12,658.5
717	9,750.0	8,808.5
138	7,500.0	6,589.5
135	11,700.0	11,312.0

TABLE 8

CHEMICAL ANALYSES OF FECES AND OF BUR CLOVER NO. 3

Feces	Dry matter per cent	Crude protein per cent	Nitrogen-free extract per cent	Ether extract per cent	Crude fiber per cent
Sheep No. 137	41.78	5.98	16.90	1.60	12.62
Sheep No. 139	38.53	5.57	15.72	1.45	11.49
Sheep No. 717	45.02	7.08	18.39	1.82	12.41
Sheep No. 138	46.43	6.69	18.69	1.77	13.99
Sheep No. 135	41.99	5.96	17.06	1.53	12.51
Bur Clover No. 3	91.15	16.28	40.87	2.02	25.04

TABLE 9

COEFFICIENTS OF DIGESTIBILITY OF BUR CLOVER NO. 3

Sheep No.	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber
137	56.90	65.46	61.12	25.51	52.61
139	55.41	63.91	59.43	24.28	51.60
717	55.38	60.71	59.35	18.60	55.23
138	55.25	63.90	59.82	23.02	50.91
135	55.46	64.60	59.64	26.77	51.70
Average	*55.68*	*63.72*	*59.87*	*23.64*	*52.41*

Tables 3, 6, and 9 show the percentage of each ingredient in the three lots of bur clover digested by the animals. As separate data were obtained from each animal, the average represents the results of five separate trials. The variation of individual sheep from the average of each trial was very small. The greatest variation is found in the percentage of ether extract digested, which is the nutrient present in smallest amounts and is therefore subject to the greatest amount of experimental error. The variation of the ether extract from the average is not very great and has little influence upon the total digestible nutrients in the feed.

There was some variation in the moisture content of the three lots of bur clover, and therefore a comparison can best be made upon the dry basis. The chemical composition of the three lots on the dry basis is given in table 10.

TABLE 10

PERCENTAGE COMPOSITION OF BUR CLOVERS 1, 2, AND 3; DRY BASIS

Bur Clover No.	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	Total ash	CaO	P_2O_5
1	17.71	46.93	3.34	22.97	9.05	.....	0.72
2	17.35	46.27	2.54	25.11	8.72	1.24	0.68
3	17.86	44.84	2.22	27.48	7.61	1.30	0.67

The difference in composition between bur clover No. 1 and No. 2 does not appear very significant except for the lower ether extract and the slightly higher crude fiber in No. 2. Perhaps the processes involving the change in color of the chlorophyll and loss of aromatic compounds may have affected the amount of ether-extractable material.

Bur clover No. 3 is slightly higher in protein than No. 1 and lower in nitrogen-free extract, ether extract, and total ash. The decrease in nitrogen-free extract and ash, with the corresponding increase in crude fiber, may be taken as indicative of leaching.

The average coefficients of digestibility of each nutrient in the three lots of bur clover are shown in table 11.

TABLE 11

AVERAGE COEFFICIENTS OF DIGESTIBILITY

Bur Clover No.	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber
1	64.20	74.11	70.91	56.89	51.51
2	59.18	68.01	64.14	40.85	50.66
3	55.68	63.72	59.87	23.64	52.41

Table 11 shows that from bur clover No. 1 to No. 3 there was a progressive decrease in digestibility of all nutrients except crude fiber.

The extent to which the decrease in digestibility could be attributed relatively to leaching by rain or to changes resulting from other factors was not known, since the difference in chemical composition was not very great. It is possible, however, that considerable amounts of each nutrient, with the exception of crude fiber, might have been extracted and still not have changed very greatly the composition of the residue. Since part of the soluble material had already been removed from bur clovers 2 and 3, it was expected that if samples

of all three lots were subjected to leaching under identical conditions in the laboratory that the difference in amount of material extracted would indicate the extent of the loss by rain, providing other factors such as exposure to sunlight and air had not changed the solubility of the nutrients. Accordingly, approximately 400-gram samples each of bur clovers 1, 2, and 3 were taken for the leaching experiments. The burs were separated from the stems and leaves, and the percentage of each was determined. The percentage of burs was 31.4 per cent, 30.8 per cent, and 31.2 per cent for samples 1, 2, and 3, respectively. The stems and leaves were thoroughly mixed and divided into two approximately equal portions. Each sample was then made up to exactly 30 per cent burs and 70 per cent leaves and stems. One portion was used for analysis, the other was leached. The weights of samples leached were 172 grams, 168 grams, and 182 grams respectively, for bur clovers 1, 2, and 3. The samples were placed in soil percolators for one hour with two liters of distilled water; they were then washed twice with one-liter portions of water, and the final volume of extract was made up to four liters. The extract was first filtered with suction through linen, and the portions used for analysis were filtered through filter paper to remove any solids in suspension. The percentage of the total dry matter extracted was determined and found to be 19.94, 15.97, and 11.73 for bur clovers 1, 2, and 3, respectively.

In order to ascertain whether exposure to sunlight and air without leaching would bring about chemical changes which would decrease the amount of soluble material, a quantity of bur clover was collected and dried by spreading out in a thin layer on canvas for 2½ days. One-half was then stored and the other allowed to bleach in the sun for 40 days. It was protected against loss of leaves by screens and was taken indoors when the weather was inclement. At the end of this time it was very dry and thoroughly bleached. Duplicate 100-gram samples of each lot were then leached under identical conditions. No difference was found in the amount of total solids extracted.

In another experiment in which samples of alfalfa meal were extracted with water after exposure to irradiation from a quartz mercury vapor lamp for 2 hours at a distance of 18 inches, no difference in water soluble material was found. It was therefore concluded that exposure to light and air did not effect the solubility of the nutrients in forage and that the difference found between the different lots of bur clover was caused by the previous leaching by rain.

The difference in digestible organic matter per 100 pounds of dry matter between bur clover No. 1 and No. 2 was 4.89 pounds. The difference between No. 1 and No. 3 was 6.95 pounds. The amount of organic matter indicated to have been lost from bur clover No. 2 and No. 3 through the action of rain was 3.2 and 6.5 pounds, respectively. If this soluble organic matter is assumed to be highly digestible the greater part of the difference in digestibility can be accounted for by the loss of these soluble constituents.

The digestible nutrients in 100 pounds of dry matter in bur clovers 1, 2, and 3 are shown in table 12.

TABLE 12

POUNDS OF DIGESTIBLE NUTRIENTS IN 100 POUNDS OF DRY MATTER

Bur clover No.	Crude protein	Carbohydrate	Fat	Total*	Nutritive ratio
1	13.13	45.11	1.89	62.49	1:3.68
2	11.80	42.40	1.04	56.54	1:3.79
3	11.41	41.25	0.52	53.83	1:3.72

* Total includes fat times the factor 2.25.

The total digestible nutrients decreased from 62.5 in bur clover No. 1 to 56.5 and 53.8 in bur clover No. 2 and No. 3, respectively. This represents a decrease in total food value of 9.54 per cent in No. 2 and of 13.8 per cent in No. 3, compared to bur clover No. 1. The ratio of protein to carbohydrate and fat remained practically unchanged and is relatively narrow.

Bur clover No. 2 and No. 3 were apparently less palatable to the sheep than was bur clover No. 1. Upon changing from the latter to No. 2 it was found necessary to reduce slightly the quantity fed in order to induce the sheep to consume the entire ration.

In spite of a significant decline in total digestible nutrients, bur clover No. 3 was still comparable in digestible composition to average alfalfa hay.

Since the bur clover was cured on concrete floors, where it was possible to recover all of the burs, stems, and leaves, each lot was representative of the entire plant as it occurred in the field. The chemical composition of the burs as compared with the stems and leaves is shown in table 13.

With the exception of the ash there is no very significant difference in the composition of burs and of stems and leaves. It would, therefore, seem doubtful that the total feed value of the burs is any greater than that of the stems and leaves combined, especially as large numbers of seeds were observed to be practically unchanged in the feces.

TABLE 13

PERCENTAGE COMPOSITION OF BURS AND OF STEMS AND LEAVES; DRY BASIS

	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	Total ash
Burs	15.12	51.42	3.33	23.78	6.35
Stems and leaves	16.33	47.11	2.36	23.81	10.39

The net energy value in therms per 100 pounds dry matter for each of the three lots of bur clover has been computed according to the method of Armsby[5] and is given below:

Bur clover No. 1—43.18 therms.
Bur clover No. 2—35.35 therms.
Bur clover No. 3—32.06 therms.

According to Armsby the maintenance requirement for a 1,000-pound steer is 6 therms of net energy daily, and the average requirement for each pound of increase during fattening is 3.25 therms. The significance of the difference in digestible composition of the three lots of bur clover may be demonstrated by a hypothetical case wherein a 1,000-pound steer eats 25 pounds of bur clover daily. The gain expected from each of the lots of bur clover has been computed and is shown in table 14.

TABLE 14

COMPUTED NET ENERGY VALUE OF THE FEED AND GAIN IN LIVE WEIGHT FROM THE CONSUMPTION OF 25 POUNDS OF DRY MATTER DAILY

Bur clover No.	Total net energy therms	Required for maintenance therms	Available for gain therms	Computed gain pounds
1	10.80	6	4.80	1.47
2	8.84	6	2.84	0.87
3	8.01	6	2.02	0.62

If the total dry matter consumed daily in each case were limited to 20 pounds, the computed gains would be approximately 0.8 pound, 0.3 pound, and no gain, respectively, for bur clovers 1, 2, and 3.

Table 14 shows that even a comparatively small change in total feed value reduces the margin of energy above the maintenance requirement so that gains are seriously affected.

The effect of excessive exposure and of rain is probably minimized in this experiment because all the burs and leaves were saved. On the range there undoubtedly would be a heavy loss of leaves because of the beating effect of the rain and because of the tendency of the leaves subsequently to become brittle, easily pulverized, and hence lost by being mixed with dirt or blown away by the wind. The loss of leaves would probably cause a marked decline in protein, ash, and

digestible carbohydrate. Table 15 from Henry and Morrison[6] shows the relative composition of alfalfa hay, leaves, and stems. The difference in composition probably holds true in a general way for bur clover leaves and stems.

TABLE 15

THE PERCENTAGE COMPOSITION OF ALFALFA HAY, LEAVES AND STEMS

	Water	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	Ash
Alfalfa hay	8.6	14.9	37.3	2.3	28.3	8.6
Alfalfa leaves	6.6	22.5	41.2	3.4	12.7	13.6
Alfalfa stems	5.6	6.3	27.9	0.9	54.4	4.9

Table 15 shows that the alfalfa leaves contained 22.5 per cent protein as compared to 6.3 per cent in the stem. The leaves were also much higher in easily digestible carbohydrate and very much higher in ash. This indicates that any condition which results in loss of leaves would cause a decided decrease in forage value.

SUMMARY

Bur clover, in common with other legumes, is rich in protein and has a narrow nutritive ratio. Even when cut in advanced stages of maturity it has a higher coefficient of digestibility than most hays.

Weathering of bur clover, which included exposure to rain, resulted in a decrease in digestibility of each nutrient except crude fiber. Evidence has been presented which indicates that the loss of soluble constituents caused by rain may have been responsible for the greater part of the decrease in digestibility.

The bleaching and leaching processes apparently decreased the palatability of the bur clover used in the digestion experiments.

The significance of the decrease in digestibility on gains in live weight has been discussed in the text.

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THE EFFECT OF LEACHING ON THE NUTRITIVE VALUE OF FORAGE PLANTS[1]

H. R. GUILBERT [2] S. W. MEAD,[3] AND H. C. JACKSON[4]

INTRODUCTION

A significant decrease in digestibility of bur clover after exposure to sunlight and rain has been reported in the first paper and evidence was presented which indicated that the greater part of the decrease could be accounted for by the loss of soluble constituents through the action of rain. Field observations support these findings.

After late rains on cured range feed, cattle have been observed to cease gaining and to require supplemental feeding in order to fatten sufficiently to be marketable. Under such conditions it is difficult, in many cases, even to maintain breeding stock. This situation prevailed over a large area of California in 1929. Extensive supplemental feeding was required in many areas to fatten the cattle for beef, and stock cattle, generally, suffered from the poor feed. Among the abnormal conditions reported in cattle from some areas were pica, particularly bone craving, deformed calves, difficult parturition, and retained placenta. Many ewes which apparently were unable to lactate abandoned their lambs. The indications are that these troubles were directly associated with the poor quality of the feed.

Rain followed by warm, humid weather is favorable to the development of molds. Frequently, however, the feed dries quickly with little or no molding; and yet deterioration has occurred, as evidenced by the condition of livestock. Field observations therefore indicate that the leaching effect of rain may be the most important factor in the loss of nutritive value.

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REVIEW OF LITERATURE

Wolff[1] in Germany reported that 20 per cent of the dry substance of hay may be lost by simply soaking in cold water, and that clover hay suffers from rain more than meadow hay because from 25 to 40 per cent of its dry substance may be dissolved. He reported analyses by Ritthausen at Möckern on two samples of clover hay which were cut at the beginning of the flowering period from the same field at the same time. One was quickly dried and the other left to lie for two weeks exposed to intermittent rains. The percentage composition was reported as follows:

	Water	Crude protein	Nitrogen-free extract and fat	Crude fiber	Ash
Not rained upon	16.0	14.6	36.1	25.3	8.0
Rained upon	16.0	15.8	23.4	37.4	7.5

The principal change was a decrease in the most soluble carbohydrates and ash and a resultant increase in crude fiber.

Headden[2] of the Colorado Experiment Station reports the percentage composition of alfalfa hay before and after being exposed to rain as follows:

	Protein	Nitrogen-free extract	Crude fat	Crude fiber	Ash
Not rained upon	18.71	38.71	3.94	26.46	12.18
Rained upon	11.01	33.64	3.81	38.83	12.71

The latter hay was damaged by three rains at intervals of two days or more. The author states: "The mechanical loss of leaves and stems would tend to change the composition of the hay in the direction indicated by the analyses, but for good reasons we do not consider this to enter largely into this particular case, but attribute the changes in composition to the action of heat and moisture."

Henry and Morrison[3] state: "Exposure to the sun reduces the palatability by bleaching and causes a loss of aromatic compounds, dew works injury and rain carries away the more soluble portions. The actual damage from rain is even greater [than analysis shows], for the nutrients lost were those most soluble and hence most easily digested." According to Piper[4] the destruction of the green chlorophyll by sunlight is increased by the action of dew. He also makes the following statement:

"Westgate sprinkled perfectly cured crimson clover hay with water to imitate rain for one hour on each of three successive days. On analysis it was found in comparison with a sample unsprinkled to have lost about three-fourths of its sugar, one-ninth of its protein and three-fourths of its ash constituents."

Le Clerc and Breazeale[5] harvested a sample of greenhouse barley at the heading period and subjected the whole plant to leaching. They state: "The plant was placed in a large evaporating dish and soaked with water for several minutes. After drying, this operation was again repeated. The plant was then dried and analyzed. The washings were also analyzed, the results showing that 1.6 per cent of the whole nitrogen of the plant was lost on washing or soaking, 36 per cent of the phosphoric acid, 65 per cent of the potash, 52 per cent of the soda, 45 per cent of the magnesia, and 75 per cent of the chlorin."

Various other plants, among them rice, wheat, apple twigs, oats, and potatoes, were experimented upon in a number of ways and at different stages of growth. The largest loss of nutrients by leaching was found to occur when the plant was at maturity. In the growing state, however, some losses occur. When wheat plants were in bloom, the amounts washed out of the straw and leaves were as follows: "Nitrogen, 1.4 per cent; phosphoric acid, nothing; potash, 4.4 per cent; soda, 12.7 per cent; lime, nothing; magnesia, 10.3 per cent; chlorin, 7.6 per cent." From this Le Clerc and Breazeale state: "It is not contended that the green plants give off very much of their plant food by such treatment, for it is very probable that most of the ash ingredients removed by washing are those which were in the dead or wilted tissue, as it is well known that when plants dry or wilt, the inorganic constituents exude to the surface, where they may be easily washed off if subjected to the action of rain, dew, etc. As illustrative of this, an experiment made with freshly cut grass showed that when the grass was dried previous to treatment with water a much larger amount of ash materials was washed out. This explains why it is that when freshly cut hay has been rained upon it is only slightly injured, whereas if the rain comes after the hay has been dried the loss is considerable, sometimes as much as half of the ash ingredients being thus removed."

Digestion experiments[6] and field observations indicate that the leaching effect of rain is an important factor in loss of nutritive value. Although the effect of leaching is indicated in the literature cited, these experiments do not appear directly applicable to range conditions. Experiments were therefore undertaken with species of forage

plants common to California ranges for the purpose of showing the extent and character of the losses which could be caused by rain, and thus contribute further information on the reasons for the observed deterioration in nutritive value.

LEACHING EXPERIMENTS WITH BUR CLOVER

A quantity of bur clover was cut at an advanced stage of maturity but was still green in color. It was dried in the sun for one day in a thin layer and then cured for 6 days in cocks. This was the lot designated as No. 1, in the digestion experiments reported in the first paper.

For the leaching experiment a sample weighing 172 grams was placed in a soil percolator for one hour with two liters of distilled water; it was then washed twice with one-liter portions of water, and the final volume of the extract made up to four liters. The extract was first filtered with suction through linen, and the portion used for analysis was filtered through filter paper to remove any solids in suspension. The results are summaried in table 1.

TABLE 1

PERCENTAGE OF CHEMICAL CONSTITUENTS EXTRACTED FROM BUR CLOVER NO. 1; DRY BASIS

Crude protein	16.20
Nitrogen-free extract	28.32
Calcium	30.43
Phosphorus	45.71
Chlorine	86.02
Total silica-free ash	59.11
Total solids	19.94

As shown in table 1, nearly 20 per cent of the dry matter of the forage was extracted by water. The silica-free ash, the various ingredients of the ash, and the nitrogen-free extract were most susceptible to leaching. Although the percentage loss of protein was least, the amount extracted appears significant particularly since the digestion experiments show a decreased availability of the protein after exposure to weathering and to rain.

Although the nutritive value of bur clover is markedly affected by rain, it is still much higher than that of the dried grasses and other forage under similar conditions; and stock can be maintained fairly

well so long as the clover burs are obtainable in adequate quantities. The relative effect of leaching on the burs and the enclosed seeds as compared with the remainder of the plant is, therefore, of interest, and another experiment was carried out to determine this.

A sample from another lot of bur clover was used in this experiment. The burs were separated from the stems and leaves, and samples of each were thoroughly mixed and quartered, opposite quarters being taken for leaching and analysis. The samples for leaching were weighed and placed in soil percolators, which were then filled with distilled water and allowed to stand for approximately one hour. The extract was then drawn off, and the residue washed with distilled water until it came off practically colorless. The extract was then made up to the nearest convenient volume. A portion of each of the original samples and of the extracts were analyzed. The results are shown in table 2.

TABLE 2

PERCENTAGE OF CHEMICAL CONSTITUENTS EXTRACTED FROM CLOVER BURS AS COMPARED TO STEMS AND LEAVES; DRY BASIS

	Clover burs	Leaves and stems
Crude protein	9.3	11.2
Nitrogen-free extract	15.3	35.3
Calcium	9.5	19.6
Phosphorus	16.3	58.7
Total silica-free ash	26.5	34.4
Total solids	10.8	21.7

The clover burs were much more resistant to leaching than the stems and leaves, the loss of total solids being only half as great. The difference in the percentage of calcium, phosphorus, and carbohydrate extracted is especially large.

LEACHING EXPERIMENT WITH OAT HAY

A sample of good quality red-oat hay was cut into 2 to 3-inch lengths, and a weighed quantity placed in a soil percolator with distilled water and allowed to stand for approximately one hour. The extract was then drawn off and the residue washed with distilled water until it came off practically colorless. The extract was filtered and made up to a convenient volume. Samples of the original, of the residue after leaching, and of the extract were analyzed.

The percentage of chemical constituents extracted is shown in table 3.

TABLE 3

PERCENTAGE OF CHEMICAL CONSTITUENTS EXTRACTED FROM RED-OAT HAY; DRY BASIS

Crude protein	1.1
Nitrogen-free extract	14.2
Calcium	31.3
Phosphorus	21.4
Chlorine	67.2
Total silica-free ash	59.2
Total solids	10.4

The loss of total solids was less than from bur clover under similar conditions of extraction. The oat hay was lower in protein, and a small amount was removed by leaching. The percentage of carbohydrate soluble in water was also less than in the clover. The loss of ash, however, was nearly 60 per cent of the total.

LEACHING EXPERIMENTS WITH NATURALLY-CURED RANGE FORAGE

All of the previous experiments were conducted on samples cut in the green stage and dried. Because changes in composition occur during the latter stages of maturity, it appeared desirable to experiment with samples of naturally-cured range feed. The following is a description of the samples used:

Sample No. 200 was soft chess (*Bromus hordeaceus*). It was dry and bleached, and the seeds were mostly shattered. The sample was collected on June 3, 1930.

Sample No. 212 was a composite in which stork's-bill alfilaria (*Erodium botrys*) predominated. It was collected on June 5, 1930; it was dry and bleached, and the seeds were completely shattered.

Sample No. 215 was dry, bleached bur clover (*Medicago hispida*). Many of the burs had fallen to the ground, but a large percentage of these were included in the sample in order to have it as nearly representative of the material grazed as possible. It was collected on June 9, 1930.

All the samples were ground to pass through a 40-mesh screen. Fifty-gram samples of each were placed in flasks with 500 cc. of distilled water and allowed to stand for approximately one hour. The

extracts were filtered with suction and the residues washed with 150 to 200 cc. of distilled water. The final volume of extract varied from 500 to 590 cc. The purpose of the experiment was to ascertain whether or not the relative loss of the various nutrients would be similar to that found in previous experiments.

The percentage of each nutrient extracted is shown in table 4.

TABLE 4

PERCENTAGE OF CHEMICAL CONSTITUENTS EXTRACTED FROM NATURALLY-CURED FORAGE; DRY BASIS

	No. 200, soft chess	No. 212, alfilaria	No. 215, bur clover
Crude protein	18.2	12.0	12.9
Nitrogen-free extract	12.1	12.6	15.0
Calcium	30.5	11.8	9.5
Phosphorus	37.0	45.4	31.9
Chlorine	72.6	63.0	76.9
Total silica-free ash	62.7	28.3	32.8
Total solids	10.7	8.0	10.6

The greatest percentage loss was in the ingredients of the silica-free ash, a fact which is in agreement with the other experiments. The percentage loss of ash from soft chess was approximately double that from alfilaria and bur clover. Compared with all the previous experiments, a higher percentage of protein relative to other ingredients was extracted.

Another lot of four naturally-cured samples, each weighing 25 grams, was prepared in the same way as the previous samples and extracted with distilled water for 5 hours in a Soxhlet apparatus. At the end of this time the water which came over was colorless. The treatment of these samples probably approached complete extraction. A brief description of the samples follows:

No. 221. Soft chess, dry, bleached, and the seeds partly shattered.

No. 222. Wild oats, dry, bleached, and the seeds mostly shattered.

No. 226. Bur clover, dry, bleached, and consisting largely of burs and some stems. Most of the leaf material had been shattered and lost.

No. 228. Stork's bill alfilaria, dry, bleached, and the seeds shattered.

All of the samples were collected in the same locality on June 10, 1930. They were taken from a different area than the samples reported in table 4, and had been dry somewhat longer. The results of the Soxhlet extraction are shown in table 5.

TABLE 5

PERCENTAGE OF CHEMICAL CONSTITUENTS EXTRACTED FROM NATURALLY-CURED FORAGE WITH SOXHLET APPARATUS; DRY BASIS

	No. 221, soft chess	No. 222, wild oats	No. 226, bur clover	No. 228, alfilaria
Crude protein	6.8	11.8	14.3	12.2
Nitrogen-free extract	6.1	8.9	15.6	17.1
Calcium	26.3	47.2	10.2	11.7
Phosphorus	18.8	24.6	27.7	42.5
Total silica-free ash	30.0	66.9	34.8	24.6
Total solids	5.0	8.3	11.9	12.1

The loss of total solids varied from 5 to 12 per cent, and the greatest percentage loss was in the silica-free ash. There was a relatively greater loss of calcium than of phosphorus in wild oats and soft chess, whereas the reverse was found in bur clover and alfilaria. The variation in chemical composition between this lot of samples and the first lot of naturally cured forage, may account for some of the variations in leaching. The soft chess sample, No. 221, for example, was significantly lower in protein and nitrogen-free extract and higher in fiber than sample No. 200. The general trend of the results is the same as in previous experiments.

The results of this experiment indicate that the lower amount of total solids extracted in the range samples as compared to bur clover No. 1 results not so much from the method of leaching as from a lower content of soluble material. The loss of total solids in the wild oats and in soft chess, sample No. 200, was not far from that found in red-oat hay, table 3. The loss from bur clover sample No. 226 was similar to that found for a pure sample of burs, table 2. Because of the shattering of leaves under field conditions, the bur clover and alfilaria samples used in these experiments are not representative of the entire plant. It is not possible, therefore, from the data available, to compare directly the solubility of nutrients in forage cut in advanced stages of maturity but still green, with the fully matured and naturally dried forage.

THE COMPOSITION OF RESIDUES AFTER LEACHING

The foregoing data have shown the percentage loss of the various nutrients through leaching and hence indicate the possible loss in tonnage of cured feed. The utilization of the material which is left, however, is a most important consideration. The composition of the residues are shown in tables 6, 7, and 8.

TABLE 6

COMPARISON OF THE PERCENTAGE COMPOSITION OF UNLEACHED PORTIONS OF BUR CLOVER AND RED-OAT HAY WITH THE RESIDUES AFTER LEACHING; DRY BASIS

	Bur clover No. 1*		Bur clover burs		Bur clover stems and leaves		Red-oat hay	
	Unleached sample	Leached residue	Unleached sample	Leached residue†	Unleached sample	Leached residue†	Unleached sample	Leached residue
Crude protein	16.86	17.77	15.12	15.38	16.33	18.51	5.15	5.62
Nitrogen-free extract	45.54	41.67	52.21	49.73	46.93	38.76	60.35	57.68
Ether extract	3.31	3.94	2.54	2.85	2.54	3.24	3.43	3.83
Crude fiber	25.31	31.25	23.78	26.66	23.81	30.41	25.00	28.22
Silica-free ash	7.56	3.89	5.84	4.81	9.53	7.98	3.07	1.08
Acid-insoluble ash	1.42	1.48	0.51	0.57	0.86	1.10	3.00	3.57
Calcium	0.95	0.94	0.83	0.84	1.20	1.23	0.23	0.18
Phosphorus	0.30	0.22	0.40	0.38	0.20	0.10	0.18	0.16
Chlorine	0.60	0.20	------	------	------	------	0.76	0.29

* Results in the first two columns were obtained from a different sample from that reported in table 1.

† Computed from the original and the extract.

Nitrogen-free extract represents a large percentage of the total dry matter in the plant. In the leaching experiments shown in table 6 there was a considerable loss of this constituent, which has the effect of increasing the percentage of other ingredients of the residue in which the percentage loss is less.

The protein in the residue after leaching was in every case higher than in the original material. The nitrogen-free extract was lower in the residue in every case, while the ether extract and crude fiber were higher than in the unleached sample. The silica-free ash was reduced in every instance, and in the oat hay it was reduced to a little more than 1 per cent, which is extremely low.

Of the ingredients of the ash, calcium was least affected by leaching except in red-oat hay, where the calcium loss was greater than that of phosphorus. The phosphorus of bur clover No. 1 was reduced from 0.30 per cent to 0.22 per cent. In the case of the bur clover leaves and stems, the amount was reduced to 0.10 per cent, which is definitely low. Animals grazing on leached bur clover in which the per cent of burs eaten is less than in these samples may be ingesting less than optimum amounts of phosphorus.

The ratio of calcium to phosphorus in bur clover No. 1 was changed from approximately 3:1 to 4.5:1, while in the stems and leaves it was changed from 6:1 to 12:1. The clover burs contained twice as much phosphorus as the stems and leaves, and the ratio of calcium to phosphorus was not affected by leaching. The quantity of burs available may thus have a distinct bearing on the nutrition of animals grazing on dried range feed which has been subjected to rain.

The composition of the unleached material and of the residue after leaching of the samples of naturally-dried range feed is given in tables 7 and 8. The residues were not analyzed in this case but have been computed from the analyses of the original and of the extract.

TABLE 7

COMPARISON OF THE PERCENTAGE COMPOSITION OF THE UNLEACHED PORTIONS WITH LEACHED RESIDUES OF NATURALLY-CURED FORAGE; SILICA AND MOISTURE-FREE BASIS

	No. 200, soft chess		No. 212, alfilaria		No. 215, bur clover	
	Unleached sample	Leached residue	Unleached sample	Leached residue	Unleached sample	Leached residue
Crude protein	9.43	8.64	5.74	5.49	15.02	14.62
Nitrogen-free extract	60.44	59.55	53.33	51.41	43.19	41.04
Ether extract	1.69	1.89	2.65	2.88	1.91	2.14
Crude fiber	25.70	28.78	34.68	37.70	33.37	37.31
Silica-free ash	2.74	1.14	3.60	2.52	6.51	4.89
Calcium	0.36	0.28	1.27	1.23	1.26	1.27
Phosphorus	0.27	0.19	0.11	0.07	0.25	0.19
Chlorine	0.22	0.07	0.27	0.11	0.39	0.10

Table 7 shows that the protein and nitrogen-free extract of the residue is lower than that of the original samples, and the ether extract and crude fiber is greater. The most significant change was in the amount of silica-free ash. The calcium content of soft chess was reduced by leaching, but there was no appreciable change in the calcium content of alfilaria and bur clover. In all samples the phosphorus was lowered and the chlorine greatly reduced.

TABLE 8

COMPARISON OF THE PERCENTAGE COMPOSITION OF THE UNLEACHED PORTIONS WITH LEACHED RESIDUES OF NATURALLY-CURED FORAGE (SOXHLET EXTRACTION); SILICA AND MOISTURE-FREE BASIS

	No. 221, soft chess		No. 222, wild oats		No. 226, bur clover		No. 228, alfilaria	
	Unleached sample	Leached residue	Unleached sample	Leached residue	Unleached sample	Leached residue	Unleached sample	Leached residue
Crude protein	6.78	6.65	4.56	4.39	18.23	17.74	5.24	5.23
Nitrogen-free extract	55.29	54.67	59.92	59.50	46.80	44.79	54.91	51.78
Ether extract	2.39	2.52	1.85	2.02	3.63	4.12	2.82	3.21
Crude fiber	31.54	33.21	30.05	32.78	25.71	29.18	28.72	32.65
Silica-free ash	4.00	2.95	3.62	1.31	5.63	4.17	8.31	7.13
Calcium	0.24	0.19	0.29	0.17	1.20	1.23	1.81	1.82
Phosphorus	0.25	0.21	0.13	0.11	0.30	0.25	0.08	0.05

In agreement with the previous experiments, table 8 shows that the greatest change was in the amount of silica-free ash. The phosphorus was lowered in the residues of all the samples; the calcium was reduced in wild oats and soft chess; but the percentage remained practically unchanged in the alfilaria and bur clover. The protein and nitrogen-free extract of the residues were slightly lower than that of the unleached samples, and the ether extract and crude fiber were greater, as was found with the other samples of naturally-cured forage.

DISCUSSION

It was recognized from the beginning that it would be impossible to conduct these experiments so that the leaching would be comparable to that resulting from a given amount of rainfall on the range. The methods which have been used are therefore purely arbitrary and intended only to show the relative losses of the various constituents in order that the reasons for the observed decrease in nutritive value might be more clearly understood.

The results of some of the experiments probably represent nearly complete extractions. Probably the amount extracted was not in excess of that sometimes occurring on the range, when the feed remains saturated for one or two days and is leached by intermittent showers totaling one to three inches or more of rainfall.

The loss of a high percentage of the silica-free ash is significant. Elliot, Orr, and Wood[7] in part II of their investigation on the mineral content of pasture grass in the British Isles concluded that there was no striking difference in the total energy value of good and poor pastures, but that wide differences existed in the proportion of the mineral constituents and that high mineral content was associated with high nutritive value.

Aside from the specific functions of inorganic elements in metabolism, the concentration of mineral salts in the intestinal tract appears to have important functions in the processes of digestion. In regard to this, Orr[8] states: "The ebb and flow of fluid between the lumen of the gut and the blood stream is controlled by the concentration of mineral salts in the intestinal contents and the membrane lining the intestines. An increased amount of mineral salts in the intestinal contents tends to cause a flow of fluid from the blood to the intestines which in extreme cases causes diarrhea." It is a common observation on the ranges during the dry season that the feces of cattle become dry and comparatively hard. There is evidence that the mineral content of the feed may be responsible.

The loss of chlorine together with that of sodium (Le Clerc and Breazeale[5]) undoubtedly accounts for the increase in salt consumption observed in cattle grazing upon forage which had been damaged by rain.

The removal of the most soluble carbohydrates and proteins may leave in the residue the more complex compounds and nutrients which are protected from water and enzyme action by cellulose walls, thus resulting in lower digestibility. The increased fiber content may also have the effect of depressing the digestibility of the other constituents,[9, 10] in addition to being itself more difficult of digestion than other forms of carbohydrate. The reduction of soluble salt and of the soluble carbohydrate may have a marked effect on palatability.

A comparison of the composition of the unleached samples and the residues after leaching does not indicate clearly the extent to which leaching has occurred. A slight increase in one nutrient in the residue might mean a considerable loss in certain other nutrients which would be shown only by an analysis of the extract. An increase of from 3 to 6 per cent in crude fiber for example, was coincident, in these experiments, with losses of from 10 to 20 per cent of total solids.

SUMMARY

The greatest percentage loss caused by leaching was of the constituents of the silica-free ash, which represents that portion of the mineral in the plant which is available to the animal. This loss varied from 25 to 67 per cent in the different samples.

Of the ingredients of the ash which were analyzed, chlorine was lost in greatest amount. The percentage loss amounted to 67 per cent in oat hay and as high as 86 per cent in bur clover. The experiments indicate that practically all of the chlorine may be leached out of dried pastures by excessive rainfall. This is in agreement with the observed salt requirements of stock after feed has been damaged by rain.

In the bur clover and alfilaria samples the percentage of calcium in the forage after leaching was not significantly different from the unleached portion. Phosphorus was distinctly lower in the leached material, particularly in the case of the bur clover leaves and stems. The ratio of calcium to phosphorus in these species thus tends to be widened by leaching. In the grass species a larger percentage of calcium was lost, and the percentage in the residue was lower than in the unleached sample. This may be significant from the standpoint of nutritive value because these dry grasses are in general probably below optimum in calcium. Since the phosphorus is also reduced, the Ca:P ratio remained practically unchanged.

The amount of nitrogen-free extract lost by leaching varied from 6 per cent of the total in a sample of dry bleached soft chess to 35 per cent in bur clover stems and leaves. This loss represents largely the sugars, which are easily digested and which may also influence palatability.

The amount of crude protein lost varied from 1 per cent of the total in oat hay to 16 per cent in bur clover and 18 per cent in soft chess. The loss of protein by leaching from the samples which were cut green and dried was relatively less than that of other constituents, so that there was a higher per cent of protein in the residue than in the original sample. In the naturally-cured samples there was a reduction in the per cent of protein in the leached residue as compared with the original sample. In general the change in percentage of protein does not appear very significant, but there may be a very

significant difference in availability of the residual material as compared with that extracted.

Ether extract is influenced only slightly by leaching. The percentage in the residue is higher than in the original material.

Crude fiber remained entirely in the residue. The decrease in other nutrients caused a very significant increase in the percentage of this material in the dry matter after leaching. An increase of from 3 to 6 per cent in crude fiber was coincident with losses of from 10 to 20 per cent of total solids. The increased fiber content may have a depressing effect upon digestibility of other nutrients in addition to being, itself, difficult of digestion.

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